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13. ABSTRACT

The major purpose of this report is to review findings from our laboratory comparing the actions of intravenously administered live E. coli organisms and E. coli endotoxin in dogs and monkeys. The preponderance of data obtained from such experiments seems to indicate that the endotoxin shock model is a valid means of eliciting many of the characteristics of shock seen in the patient. Differences are apparent between the models, however, and further research is therefore needed to perfect a more adequate experimental preparation.

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A COMPARISON OF THE RESPONSE OF CANINE AND PRIMATE SPECIES TO BACTERIAL AND BACTERIAL ENDOTOXIN

Lerner B. Hinshaw

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MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE
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Gram-negative septicemia remains a serious threat in clinical medicine in spite of the knowledge gained through multitudes of experiments carried out on animals administered endotoxin. Even though there are many claims for adequate therapy in the patient in septic shock, therapeutic trials in experimental animals under conditions of rigid controls have been relatively unsuccessful (Oklahoma Shock Tour, 1964).

Questions regularly arise concerning adequacy of the animal shock models adopted for the hopeful discovery of causative mechanisms in the human clinical entity. Serious question has arisen regarding the relevance of the canine endotoxin shock model to septic shock in man. There appears to be no animal model currently available which exactly reproduces all the pathophysiological manifestations described in clinical septic shock. The pressing need for an animal model closely approximating the clinical situation has prompted investigations in our laboratory which have compared both species responses and two means of eliciting experimental septic shock (1-7).

The major purpose of this report is to review findings from our laboratory comparing the actions of intravenously administered live E. coli organisms and E. coli endotoxin in dogs and monkeys. The preponderance of data obtained from such experiments seems to indicate that the endotoxin shock model is a valid means of eliciting many of the characteristics of shock seen in the patient. Differences are apparent between the models, however, and further research is therefore needed to perfect a more adequate experimental preparation.

General Statements Regarding Methodology.

Experiments have been carried out during the past four years on adult mongrel dogs and rhesus monkeys given LD_{60}-LD_{100} injections of live organisms or endotoxin. In some instances, animals were studied without anesthesia during an eight day post-injection period. E. coli endotoxin was obtained from Difco laboratories (Detroit). Live organisms were of the enteropathogenic Dunwald strain of Escherichia coli (type 0125:B15, Canioni). Stock cultures were maintained on tryptic soy agar transferred weekly. Tryptic soy broth was inoculated from stock culture and incubated eight hours at 37°C. Tryptic soy agar slants were inoculated from the tryptic soy broth and incubated at 37°C for 18 hours. Bacterial growth was removed from tryptic soy agar slants by washing with sterile isotonic saline solution. The suspension of organisms was then centrifuged, decanted, and resuspended in sterile isotonic saline solution. The final concentration of organisms was adjusted to 10 per cent transmittance in a spectrophotometer by dilution of the suspension with sterile isotonic saline solution. A reading of 10 per cent transmittance corresponded to a concentration of approximately $2X10^9$ organisms per millileter. For each experiment, the concentration of viable organisms was confirmed by a colony count.

Discussion of Results.

Table I shows directional changes in certain cardiovascular changes in experimental septic shock. Contrasting effects are seen between live E. coli organisms (LD_{95}) and endotoxin (LD_{95}) injections. In contrast to the typical endotoxin action in bringing about portal hypertension with hepatosplanchnic pooling and large early decreases in venous return and mean systemic arterial pressure, live organism injection elicits only a negligible change in portal vein pressure which seems undissociated with the small gradual declines in venous return and

arterial pressure. There seems to be a general overall relaxation of resistance vessels after live organism injection, while resistance changed in variable directions after endotoxin. These results in dogs with live organisms are all seen in monkeys administered endotoxin (5). They appear to suggest that pooling may be of a gradual general nature, occurring in both abdominal visceral as well as in extrahepatosplanchnic regions. It may be that the means of producing shock in dogs by injecting endotoxin leads to an exaggerated picture of hepatosplanchnic pooling as a specific response to endotoxin. Species differences seem to diminish in regard to exaggerated abdominal visceral pooling when live organisms are administered to the canine species. An alternative explanation is that endotoxin is released only slowly after live organism injection, thus producing a less intense effect on the hepatosplanchnic region. This effect, however, appears to resemble more closely that which is expected to occur in man in septic shock, as revealed in subhuman primate investigations (2,3,5).

Table Ia illustrates changes in hematocrit, pH and heart rate in dogs given either live organisms or endotoxin. Marked increases in hematocrit and decreases in pH are common observations after endotoxin in the canine species. These changes are seen in like intensity after live E. coli organism injection in quietly restrained unanesthetized dogs and are readily correlated with impending irreversibility and death (6). Early bradycardia lasting not longer than one hour followed by steadily developing tachycardia are seen in dogs after both live organism and endotoxin injection (6). The decreased heart rates however are not observed in monkeys, and their absence may be due to the absence of increased vagal tone or a more intense cardioacceleratory reflex response in the subhuman primate in the shocked state. The monkey ordinarily dies after endotoxin with no change in hematocrit, which is in

marked contrast to the hemoconcentration in dogs, but probably resembles man in this regard.

Table Ii shows the changes in renal responses to live organism and endotoxin injection in dogs. The commonly observed renal alterations in dogs given endotoxin are decreases in renal blood flow and urine flow. In contrast, live organism injection elicits renal hyperemia, renal vasodilation and polyuria in half of the animals, features totally absent with lethal injections of endotoxin. These renal responses after LD_{95} injections of live organisms are postulated to occur in the pre-stages of clinical septic shock, the so-called "warm" early phase of shock. Such events seen also after exceedingly small intravenous injections of endotoxin may be due to a steady slow release of endotoxin as the organisms are killed in the blood stream. They may serve as an important early warning of impending shock and thus may enhance the suitability of the live organism shock model in both mechanism and therapy studies.

Table IIa compares canine forelimb responses to both endotoxin (LD_{100}) and live organism (LD_{100}) injections (7). In contrast to previous studies, these experiments give support to the assumption that endotoxin may be the active component following injection of live E. coli organisms. Innervated and denervated forelimbs responded to both organism or endotoxin injection with profound vasoconstriction, seen most prominently in the small vessel segment (small artery→ small vein portion of the limb vascular bed). This study provides support for the view that circulating vasoconstrictor agents are performing a more prominent role than peripheral nerve stimuli in these two shock models. Capillary pressures were postulated to be exceedingly low on the basis of decreased perfusion pressure (systemic hypotension) and pre-capillary vasoconstriction. Limb weights fell and remained low in all studies and there was therefore no evidence

for peripheral pooling of perfusate on the basis of sequestration or extravasation into skin and muscle tissues. These results, if applied to the primate species, would suggest that extremely prolonged low peripheral blood flows in clinical septic shock may exert deleterious effects on other organ systems because of the release of metabolic and/or toxic factors into the venous effluent from ischemic skin and muscle tissues.

Tables III and IIIa present directional changes in certain hemodynamic, respiratory and metabolic parameters in rhesus monkeys administered either live E. coli organisms (>LD_{60}) or endotoxin (>LD_{60}). The profound changes observed (2) simulated observations in humans in septic shock. The only differences between organism and endotoxin injections were the rate of onset of measurable changes and the severity of hypoxia. Monkeys given endotoxin developed hypotension, decreased cardiac output, and ventilatory changes much earlier than those given E. coli (2). The severe hypoxia observed within five minutes of infusion of endotoxin was not observed in any of the animals given E. coli; however, all monkeys in both groups demonstrated decreased arterial Po_2 or increased A-a gradients at some time during the course of the study. The profound systemic hypotension observed in both the E. coli and endotoxin groups was related to a rapidly decreasing cardiac output and a decrease in total peripheral resistance. Animals from both series demonstrated the hyperventilation, hypoxia, and increased A-a gradients described in patients with septic shock. Plasma catecholamine levels were in general minimally elevated until the second hour of hypotension and then rose modestly. The late rise in peripheral resistance seen in most animals appears to be related to the increase in catecholamine levels. These studies following infusion of live organisms reveal a time course, and pathophysiological responses, similar to the human in septic shock.

Tables IV and IVa display pulmonary changes in monkeys given lethal injections of either live E. coli organisms or endotoxin. In general, the ultrastructural alterations in the lungs were markedly similar. Pulmonary capillaries were engorged with polymorphonuclear leukocytes undergoing fragmentation fifteen minutes after injections of endotoxin or E. coli organisms. Fragmentation together with loss of specific granules (lysosomes) of the neutrophils were noted one hour post-injection. Endothelial cellular membranes appeared fuzzy and indistinct (increased capillary permeability?) at sites where polymorphonuclear leukocytes were adhering. Edema of the perivascular space was seen in all tissues examined. There was no evidence for intravascular coagulation of fibrin and platelet aggregates. These widespread morphological alterations, occurring in a similar fashion after both live organism and endotoxin injections, could explain some of the hemodynamic, respiratory and metabolic derangements reported in monkeys in shock.

Comments.

Future research will be required to further elucidate similarities and differences between the live organism and endotoxin shock models. Preliminary evaluation of the two models demonstrates notable similarities in ultrastructural hemodynamic, respiratory and metabolic alterations, although differences are observed. These differences may be important inasmuch as they may apply more closely to the human shock entity.

Use of live organisms possesses the advantage of yielding a "pre-shock", "warm" phase, which seems to offer advantages for early diagnosis and treatment of the animal model.

Similarities between the two models may suggest that the two forms of shock are identical in mechanism. This is probably true; however, similarities of responses do not necessarily prove that the mechanisms of elicitation of shock are identical. Portions of common pathways may be utilized in many forms of shock which may suggest gross similarities while subtle biochemical differences may be overlooked.

It is also possible that the infusion of live organisms is a more relevant model because it allows for the steady release of small amounts of endotoxin, thus again more closely approximating the clinical entity.

It is felt that a wise and fruitful course of action to follow in future studies is the execution of simultaneous or alternate investigations using both dogs and monkeys, as well as other species, and employing live organisms or endotoxin separately. The ultimate goal is to develop the most ideal experimental shock model, which should ultimately hasten the day of a full understanding of the mechanisms of clinical septic shock.

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Table I. CARDIOVASCULAR CHANGES IN EXPERIMENTAL SEPTIC SHOCK

Parameter → / Shock Model: ↓	Portal Vein Pressure	Venous Return	Mean Systemic Arterial Pressure	Total Peripheral Resistance
E. coli organism (dog)	Small ↑	Gradual ↓	Gradual ↓	Consistent ↓
E. coli endotoxin (dog)	Large Early ↑	Large Early ↓	Large Early ↓	Variable Change

Table Ia. CHANGES IN EXPERIMENTAL SEPTIC SHOCK

Parameter	Hematocrit	pH	Heart Rate
Shock Model:			
E. coli organism (dog)	↑	↓	Early ↓ Later ↑
E. coli endotoxin (dog)	↑	↓	Early ↓ Later ↑

Table II. RENAL CHANGES IN EXPERIMENTAL SEPTIC SHOCK

Parameter →	Renal Blood Flow	Urine Flow
Shock Model ↓		
E. coli organisms (dog)	Early ↑ or ↓	Same as Blood Flow
	Later No Δ or ↓	
E. coli endotoxin (dog)	Early ↓	Same as Blood Flow
	Later ↓	

Table IIa. SKIN AND MUSCLE CHANGES IN EXPERIMENTAL SEPTIC SHOCK

Parameter → / Shock Model: ↓	Large Vessel Resis.	Small Vessel Resis.	Venous Resis.	$\Delta R_{sv}/R_v$	Limb Weight
E. coli organisms (dog)	↑	↑↑↑	↑	↓	↓
E. coli endotoxin (dog)	↑	↑↑↑	↑	↓	↓

Table III. HEMODYNAMIC, RESPIRATORY AND METABOLIC CHANGES IN EXPERIMENTAL SEPTIC SHOCK

Finding ➡	Decreases in CO, MSAP, and TPR*	Decreases in $PaCO_2$, PaO_2	Increased A_AO_2 Gradient
Shock Model:			
E. coli organisms (monkey)	+	+	+
Endotoxin (monkey)	+	+	+

*CO = Cardiac Output
MSAP = Mean Systemic Arterial Pressure
TPR = Total Peripheral Resistance

Table IIIa. HEMODYNAMIC, RESPIRATORY AND METABOLIC CHANGES IN EXPERIMENTAL SEPTIC SHOCK

Finding →	Increases in Blood Lactate and Catecholamines (2nd hour)	Increased Minute Ventilation	Increased Heart Rate
Shock Model: ↓			
E. coli organisms (monkey)	+	+	+
Endotoxin (monkey)	+	+	+

Table IV. PULMONARY CHANGES IN EXPERIMENTAL SEPTIC SHOCK

Finding → / Shock Model: ↓	Capillary Engorgement of PMN* Cells	Fragmentation of PMN Cells	Lysosomal Breakdown
E. coli organisms (monkey)	+	+	+
E. coli endotoxin (monkey)	+	+	+

*PMN = Polymorphonuclear Leukocytes

Table IVa. PULMONARY CHANGES IN EXPERIMENTAL SEPTIC SHOCK

Finding ➡ / Shock Model: ⬇	Endothelial Cell Membrane Indistinctness	Perivascular Space Edema	Intravascular Fibrin Accumulation
E. coli organisms (monkey)	+	+	−
E. coli endotoxin (monkey)	+	+	−